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## A Modified Method for Implantation of Pluripotent Stem Cells under the Rodent Kidney Capsule.

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**Authors:** Fuli Zhu, Bin Sun, Yan Wen, Zhe Wang, Renee Ann Reijo Pera, Bertha Chen

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### Public Summary:

The existing methodology of transplanting stem cells into the kidney capsule of mice is used to determine if the cells can generate a tumor in live tissue. However, one major limitation of this methodology is that it is difficult to calculate the number of cells injected, especially when investigators are interested in examining transplantation with low cell numbers. This study describes and tests a modification to the existing methodology aimed at addressing this limitation. We found that the modified procedure for transplanting pluripotent stem cells under the kidney capsule allows for transplantation of a defined number of cells with significant reduction in error associated with cell leakage from the transplant site.

### Scientific Abstract:

Teratoma formation, the standard in vivo pluripotency assay, is also frequently used as a tumorigenicity assay. A common concern in therapeutic stem cell applications is the tumorigenicity potential of a small number of cell impurities in the final product. Estimation of this small number is hampered by the inaccurate methodology of the tumorigenicity assay. Hence, a protocol for tumorigenicity assay that can deliver a defined number of cells, without error introduced by leakage or migration of cells is needed. In this study, we tested our modified transplantation method that allows for transplant of small numbers of pluripotent stem cells (PSCs) under the kidney capsule with minimal cell leakage. A glass capillary with a finely shaped tip and an attached mouth pipette was used to inject PSCs into the rodent kidney capsule. Hg embryonic and induced pluripotent stem cells were tagged with Fluc and GFP reporter genes and divided in different cell doses for transplantation. Bioluminescence imaging (BLI) on the day of surgery showed that the cell signal was confined to the kidney and signal intensity correlated with increasing transplant cell numbers. The overall cell leakage rate was 17% and the rodent survival rate was 96%. Teratoma formation was observed in rodents transplanted with cell numbers between  $1 \times 10^5$  -  $2 \times 10^6$ . We conclude that this modified procedure for transplanting PSCs under the kidney capsule allows for transplantation of a defined number of PSCs with significant reduction of error associated with cell leakage from the transplant site.

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